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RECENT DEVELOPMENTS IN SEED TECHNOLOGY
(*Concluded*)

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RAPID METHODS OF MEASURING SEED VIABILITY

The prolonged dormancy of many kinds of seeds, as well as the long period of time required to complete a germination test of some non-dormant seeds, has naturally interested those who work with seeds in a study of other methods that might be used to quickly determine potential germinability. Furthermore, it is often desirable to know the viability of a seed lot within a few hours; hence the demand for rapid methods has become somewhat general.

The principal short-cut methods that have been investigated are (*a*) measurement of enzymatic activity of seeds, (*b*) excision of embryos and (*c*) immersion of seeds in reagents which differentiate between viable and non-viable seeds by color reaction.

Measurement of catalase activity as an indicator of seed viability represents the major work with seed enzymes. The ratio of the

catalase of poor seed to that of viable seed of the same kind was considered to express very closely the percentage germination of the seed. Davis (103) concluded that disappearance of catalase from dead seeds was sometimes slow and was dependent on such factors as temperature, moisture and aeration to which seeds are subjected before and after death. He found, however, that the catalase of dead seeds disorganized very rapidly if they were soaked for a time in warm water at a temperature not high enough to affect their viability, but at the same time the catalase activity of viable seeds frequently increased. Using this method and comparing the catalase activity of soaked and dry seeds, Davis concluded that a quick estimation could be made of the viability of dormant embryos, quickly germinating seeds and those that have a long germination period.

Since 1930 a number of workers have tested the catalase ratio method on seeds of apple, peach and cherry (171), wheat (248), several species of coniferous seeds (17), a number of field crops and leguminous seeds (316, 324, 425), and species of *Quercus* (56). A ratio of one or more has indicated high viability, and less than one has indicated low viability, but at the best it has been possible only to estimate approximate viability. With acorns of *Quercus,* measurements of catalase activity were of no practical value in interpreting either the progress of after-ripening or the germinability of the acorns.

Amylase activity in dry and soaked seeds of soybean and oat samples representing lots with high, medium and low vitality was determined by Mar. He found a high positive correlation between amylase activity and vitality in soaked seed of oats but not in dry seeds, nor in either soaked or dry soybean seed. Soybean seed was found (323) to contain amylase of a different type than occurs in seeds of cereals. Phenolase activity (102) was high in young seeds and low in old seeds with low vitality.

Determination of the relation between enzymatic activity and seed viability is of scientific interest and value, but at best it appears that only a close approximation of seed viability can be obtained. Present seed laws which require labeling in terms of percentage of viable seed preclude the use of estimated or approximate viability except in specific cases.

One of the most practical and successful short-cut methods of

determining seed viability is to excise the embryos and observe their behaviour under pre-determined conditions that favor their development. Two types of development of excised peach embryos taken together (466) compared favorably with the germination percentage obtained after 12 weeks of after-ripening at 5° C. The time required for the excision method was only seven days. Naked embryos were sterilized in 2% chlorine solution for 30 minutes and then transferred to sterilized one-half ounce bottles on two-thirds agar medium. Those with cotyledons spread at an angle of 75° and those with shoot and root growth were classed as viable. These results agreed closely with the percent germination from fall plantings under nursery conditions. Flemion (136) obtained similar results with peach seeds by placing the naked embryos in moist granulated peat moss at room temperature for ten days. She later (138) modified the method by using filter paper in petri dishes on which to place excised embryos of a variety of different types of dormant seeds. She kept them at room temperature (21° to 23° C.) and found that non-viable embryos deteriorated rapidly. Her results agreed well with actual germination tests. The method was applicable to seeds of the fringe tree which has an epicotyl dormancy. In a subsequent paper (139) she reported that the embryo excision method had been extended to 24 additional species in 11 families and that it was especially applicable to hard coated seeds or tc other types in which excision presents special problems. Presoaking, acid treatment and cracking of the outer coats were useful methods that aided in the excision procedures. Heit and Nelson applied the excision method to seeds with extremely dormant embryos and reported that ten to 14 days represented the average time required to make an approximate germination. Non-viable embryos discolored or decayed, while viable ones swelled, showed greening or spreading of the cotyledons or germinated. In all of the experiments with excised embryos the results were compared with actual germination tests which required various types of after-ripening, and the agreement was reasonably close. The excision method is apparently a reliable one when used by an experienced technician.

Immersion of seeds in solutions that give a color reaction has received considerable attention since 1925. The compounds used

have been indigo-carmine, dinitro benzene, selenium and tellurium salts, and tetrazolium chloride.

Neljubow tested several organic dyes which do not penetrate living tissue but readily enter dead tissue. The most promising one was indigo-carmine solution 1:2,000. Several investigators in recent years (235, 345) have used this dye with varying degrees of success. It appears that the relationship between the degree of staining and the viability must be worked out for each species. Shaefer-Safonova *et al.*, in tests with seeds of *Caragana arborescens* Lam., *Pinus silvestris* L. and *Picea excelsa* Link, found that it was necessary to remove the seed coats of *Caragana* and the endosperm in seed of pine and spruce. In *Caragana* seeds the tissue most commonly dead is the radicle, and if the apical cell stained, it indicated that the embryo could not produce a seedling. Since the apical cell is close to the root tip, a stain of more than a dot was taken to indicate a non-viable embryo. In pine and spruce the apical cells of the radicle were found to be one-third the distance from the radicle end of the embryo. Tskhoidze used solutions of indigo-carmine on seeds of the tung-oil tree at dilutions as low as 1:4,000 and reported that a viability test of 100 seeds could be made in two hours. Hao tested seeds of *Pinus, Picea, Pseudotsuga* and *Abies* by soaking the embryos in 0.5% and 1.0% solutions for one hour. Embryos that remained white or that had only a small blue spot at the root end were considered viable. He considered this dye superior to selenium salts because it is quicker and is not poisonous.

Gurewitsch developed the dinitro benzene method which involves immersion of the seeds in an aqueous solution of finely divided dinitro benzene for five hours at 15° to 17° C. or one hour at 42° to 45° C., after which they are placed in an aqueous ammonium solution for ten minutes. Living cells have the ability to reduce the dinitro benzene to nitro-phenylhydroxylamine which yields a purple color with NH_3. Seeds were cut near the epicotyl so that it would appear in the cross-section. The chemical entered the seed coats of wheat and rye readily, but it is poisonous and must be used with care. Viable seeds showed a dark purple-colored epicotyl. Weise confirmed the results of Gurewitsch and used the method on seeds of peas and beans with success but with failure on seeds of asparagus and fruit trees. He considered that dinitro

benzene could be used to test viability but not germinative capacity of seeds which require special treatment to induce germination.

Treatment of seeds with salts of selenium and tellurium was used by Eidmann. He observed that living cells of seeds reduced these salts to form free selenium and tellurium, respectively, which could be identified by their color. He preferred selenium salts in spite of their poisonous properties. Hasegawa tested both classes of compounds on seeds of Japanese cedar, cypress and pine, and reported satisfactory results. Natlacen used selenium ($NaHSeO_3$) solution on seven species of conifer and seven of broad leaf trees. He soaked the seeds 24 hours in water, then 28 hours in the selenium solution, and stated that the test gave the germination capacity of seed lots. Others (127, 237, 287, 396, 476), too, have tested selenium salts on different types of seeds. One of them (237) obtained results on wheat, barley, rye and oat seeds that compared favorably with actual germination tests. Wach used a 2% solution of $NaHSeO_3$ in which the seeds of *Pinus, Picea* and *Fagus* were soaked for 48 hours. Living seeds were colored red, but boiled, ground, mashed and finely cut seeds and embryos were not colored. He found that changing environmental conditions influenced the degree of coloring and that entrance of the chemical was facilitated by removal of the seed coats in many species. With conifers he noted that the endosperm was colored only if the seeds had started to germinate, which indicated that coloring occurs only when stored reserve material has formed a diastase. Amorphous selenium was first deposited in the inter-cellular spaces and the cell walls, then inside the cells of pine and beech embryos. He was unable to recommend a standard color scale for all species.

Lakon (238, 239) found the use of selenium salts distasteful because of the unpleasant odor and dangerous because of their poisonous properties. He discovered that two salts of tetrazolium in dilutions of 0.5% to 2% were satisfactory and reliable for the determination of viability of corn, barley, oat, rye and wheat seed. He used 2,3-diphenyl-5-methyl-tetrazolium chloride and 2,3,5-triphenyl-tetrazolium chloride but preferred the latter. His procedure was to soak the seeds for 18 to 24 hours, then cut them in half by a median section through the embryo so that the plumule and radicle were split longitudinally. Immediately after sectioning the halves were placed in the tetrazolium solution in petri dishes

and kept in the dark for three to four hours. The living tissues of the embryos stained orange to red color because they reduced the tetrazolium salt. He illustrated by drawings the stained area that indicated viability. He did not consider scutellar staining as absolutely essential in all cases, but in corn embryos if no staining occurred below the region from which secondary roots are produced he considered the embryo as viable and capable of producing a seedling. Absence of the stain in the plumule indicated a non-germinable seed. The results of the staining method agreed reasonably well with actual germination tests.

Using 2,3,5-triphenyl-tetrazolium chloride in solutions of 0.5% to 2%, Porter *et al.* (365) treated seeds of corn, barley, oats, wheat, rice, rye, sorghum, soybean, pea, buckwheat and Bahia grass (*Paspalum notatum*) Flugge. for different periods of time. The treatments were preceded by different periods of presoaking in water at different temperatures. A sample of seed corn with 98% to 100% germinability was tested by this staining method, and 99% to 100% of the sections gave the characteristic orange-red color. When presoaked seeds of this lot were immersed in boiling water for a five minute period and placed in the germinator, none grew, and similar seeds also remained free from stain in the tetrazolium solution. All lots of seed that were studied were tested by regular germination procedures, and the results agreed well with those obtained by the staining method. The results with seed of Bahia grass were of special interest because dehulling is a necessary procedure to insure prompt results. The sectioning and staining procedure was used after the seeds remained two to three days in water at 20° to 35° C. The results were highly significant. The value of the tetrazolium method with seeds of soybean or garden bean was recognized as questionable because of the frequent occurrence of injured plumules.

It appears on the basis of present information that the tetrazolium method is superior to others that employ dyes or reducible salts and that it has considerable practical application for a quick evaluation of certain kinds of seed, especially of the Gramineae.

Other methods that have been used to measure seed viability are plasmolysis, detection of respiration by indicators, and endosperm injury. Doroshenko and Niethammer (325) used a plasmolytic method to measure seed viability. The latter investigator used

salts of heavy metals, such as nickel, potassium, copper, zinc and cobalt in concentrations of one-quarter to one molar. After soaking seeds in these solutions long enough to cause plasmolysis he made microscopic sections of the swollen seeds and examined them. He was able to make an approximate estimation of viability by this method. Afanasiev (3) reported that if the endosperm of seed of *Magnolia acuminata* L. is injured and the seed placed under germinative conditions, the viable seeds develop a green pigment.

THE RELATIONSHIP OF HORMONES AND INHIBITORS TO SEED GERMINATION

The discovery of growth-promoting substances has resulted in their extensive use in greenhouses, nurseries and orchards. The effect of these new compounds on the germination of seeds has been investigated, but, in general, their value has been shown to be limited. Simultaneously the existence of substances in seeds which inhibit germination has received attention. A review of the more pertinent literature dealing with stimulants and inhibitors as related to seed germination is presented herewith.

Germination of seeds of some parasitic plants has been known to be dependent on the stimulating effect of substances produced by the host plant. Bartsinskii in 1934 found that sunflower roots contain a substance which stimulates the germination of the seeds of *Orobanche cumana* Wallr., a parasite on sunflower. The substance is thermostabile and water-soluble. An extract of sunflower roots applied to soil infested with the seeds of the parasite caused prompt seed germination, and sunflowers planted after the seedlings had been killed remained free from infection. Seeds of *Striga lutea* Lour. (57), which normally germinate only after exposure to a stimulant produced by one of its hosts, were partially stimulated to germinate by first incubating them on moist filter paper for 21 days at 22° C. and then placing them on filter paper moistened with a solution of allylthiourea. Partial control of parasitic plants by artificially inducing germination of their seeds in soil prior to planting of susceptible host plants thus appears possible.

Seeds of varieties of lettuce when freshly harvested have responded favorably to treatment with various substances. Certain sulfur-bearing compounds in aqueous solutions of 0.5% (453) stimulated the germination of dormant lettuce seed at temperatures

above 25° C. Thiourea was the most generally effective, but thiosemicarbazide, thioacetamide, allylthiourea, ammonium thiocyanate and potassium thiocyanate were also stimulatory. Later results with thiourea (452) indicated that the best treatment was to soak the seed in darkness for eight to ten hours at 18° C. in a 0.5% solution and then place them to germinate at temperatures between 25° and 35° C. Seed thus treated germinated 87%, untreated seed only 1%. Dormant seed (145) of the lettuce varieties White Boston, Grand Rapids, Iceberg and Black-seeded Simpson which was soaked in 0.5% and 1.0% solutions of thiourea and germinated immediately, displayed a gradual increase in germination with the lengthening of the period of dry storage between harvest and treatment. The degree of stimulation by thiourea was affected by differences in variety and age of the seed.

Injurious effects resulting from treatment of cereal seeds with formaldehyde, copper sulfate and hot water were largely overcome, according to Grace, by adding either 1-naphthylacetic acid or 3-indolylacetic acid to the disinfecting solutions. The optimum concentration of the hormones for individual varieties of cereals lay between 0.01 and five parts per million. This method may have a practical application, especially for wheat and barley seed treated with hot water to control the fungi that cause loose smuts. Landau reported that hetero-auxin at concentrations of 10^{-7} and 10^{-9} stimulated the germination of seeds of oat, French beans, tomato and radish. Thiourea (43) had no effect on dormant barley seed but suppressed mold growth, the presence of which interferes with an estimation of germinability. Molds and common seed-borne fungi of peach (467) were reduced by treating the seed with thiourea, and seed dormancy of the Lovell variety was overcome by the treatment. Seeds of endive were stimulated to germinate by thiourea at high temperatures (451). Colchicine, which is used extensively as a practical seed treatment for the production of polyploid plants, was found by Bond to stimulate germination of seeds of *Petunia axillaris* BSP. The percentage germination was increased and the germination time reduced by soaking the seeds in different concentrations, the optimum being a 0.04% solution.

Popular interest in the culture of orchids has been stimulated by the discovery of methods that promote seed germination. Mariat used a symbiotic, jellied and sugared Knop solution to which he

added 2.6 mgs. of vitamin B_1 per liter of solution. Seeds of *Cattleya* orchids placed in this solution gave 42% of the embryos with leaves within three months, as compared with only 3% when no vitamin was added. Meyer added the juice of vine-ripened tomatoes to solid agar on which seeds of the orchid genera *Laelia, Cattleya, Epidendrum, Enyclia* and *Rodrigiasa* were germinated. Seedling development was greater on this than in the Knudson medium, and pH adjustment was unnecessary if the medium was sterilized the same day the juice was prepared. The formula for preparing the medium consisted of 250 mls. of tomato juice and water each and 7.5 grams of agar sticks.

In contrast to the stimulatory effect of growth-promoting and other substances on seed germination, a number of workers have found little or no beneficial effect and in some cases injury from the use of various substances in certain concentrations. The compounds used with little or no benefit on a great variety of plant seeds include ascorbic acid, colchicine, Hormodin "A", indoleacetic acid, indolebutyric acid, indol-3-acetic acid, K a-naphthalene-acetate, lacto flavin, levulinic acid, naphthalene acetamide, naphthalene acetic acid, thiourea, vitamin B_1 and 13 commercial hormone dusts. The kinds of seeds that were tested included tomato (98), wheat and soybeans (499), oats and broad bean (196), sugar beet (331), rice, barley and corn (341), seeds of many other agricultural crops (108, 230), corn (228), rice, corn, cotton, sorgo and soybean (19), seeds representing field crops, vegetable, grasses and flowers (27), vegetables of many kinds (326, 339), 37 types of herbaceous plants (63) and 41 varieties of woody perennials (443). The treatments were applied as liquids and dusts in various concentrations. The list of treated seeds included both dormant and non-dormant types, and in most cases plantings were made in the field to observe plant growth and yield. In all these experiments significant increases in germination were rare; generally germination was the same or less than from untreated seed.

The data that have been published relative to the effect of growth-promoting and other substances on dormant and non-dormant seeds of many kinds of plants indicate that their value is limited to a very few special types, such as dormant lettuce seed, injured cereal seeds and seeds of orchids and some parasitic plants. Until

more data to the contrary are accumulated it appears that general use of these substances is not warranted.

One of the earlier reports of a possible inhibitor to seed germination was made by Sagaguchi in 1930, who found that peach seeds in ripened fruits failed to germinate. Scott *et al.* found that fermented peach juice contains a volatile, ether-extractable substance which can destroy seed viability. Seeds that remained in rotting pulp for ten days or in fermented juice for seven days were incapable of germination. Reinhard reported in 1933 that the juice of ripened tomato fruits contains a substance which strongly inhibits germination of tomato seed and displays a less pronounced effect on lens seeds. Tomato juice was found (338) to inhibit the germination of seeds of sunflower, lettuce, carrot, cabbage and endive as well as tomato. The juice of fruits of *Cucumus sativus* L. and of *Carica papaya* L. had a similar effect. A substance (263) was isolated from the cultures of *Fusarium vasinfectum* Atkinson and *F. lycopersici* which depressed or inhibited the germination of seeds. This substance was found to be one of the primary amino compounds, thermostabile and non-specific. Water-soluble nitrogen compounds in sugar beet seed balls (441), from which ammonia is hydrolized, exhibited an injurious effect in the early stages of germination. Washing or presoaking removed the substance.

Freshly harvested lettuce seed was reported to contain a substance (417) which diffuses into the sub-stratum in which the seeds are germinated. This substance inhibited the germination of seeds placed subsequently in the same substratum. The effect appeared to be one of secondary dormancy. Non-dormant lettuce seed was made dormant (332) by immersing the seed for 24 hours at 18° to 20° C. in 25 parts per million of coumarin solution. The inhibitive effects of coumarin was overcome by the use of methods which break dormancy, namely, exposure to light, pre-chilling and treatment with thiourea.

The presence of an inhibitory substance (373) in the endosperm of freshly-harvested iris seed was found to be responsible for the pronounced dormancy. If the seed embryos were in contact with only a small portion of endosperm the inhibitory effect was evident. The inhibitive substance was removed by leaching the seeds in running water. Similarly the pronounced dormancy in seed of certain strains of cabbage (80) was shown to be caused by the presence of a

substance in the seed coats. This substance was present in the water-soluble fraction of alcoholic extracts from the seed coats. Immersion of seed for one minute in H_2SO_4 at 4° C. followed by washing in cold, running water and finally by soaking for several hours before planting removed the inhibiting substance.

In contrast to the presence of inhibitive substances in freshly-harvested seeds, old seeds of *Oenothera berteriana* Spach. with low vitality were found to contain a substance which inhibited the germination of highly viable seeds (408). This phenomenon was demonstrated by treating fresh seeds with extract or mash from old seeds.

RECOGNITION OF THE IMPORTANCE OF SEED-BORNE ORGANISMS IN SEED-LABORATORY PRACTICE

Seed transmission of plant pathogens has been recognized by plant pathologists as an important factor in the dissemination of diseases for at least 75 years. A considerable body of information has been published during that time relative to the manner in which pathogens are carried. Seed laboratories have been established in North America and Europe for 50 to 75 years, yet little attention was given to the pathological condition of seed as one aspect of seed quality until the past 15 years or more. There were at least three important reasons for this lag in the application of pathological techniques to the practice of seed testing, namely, (*a*) uncoordinated effort in agricultural experiment stations between phytopathologists and those responsible for the organization and development of seed laboratories, (*b*) lack of training and experience in the field of phytopathology among seed analysts, in general, and (*c*) inadequate methods of detecting the presence or evaluating the significance of organisms carried by seeds. Since 1930 there have been two important developments, (*a*) an active research program in a few centers which has been concerned primarily with practical methods for determining the presence of seed-borne organisms and their effect on seed germination, and (*b*) compilation of lists of seed-borne parasites together with the discovery of new ones not hitherto reported.

Detection and Importance of Seed-Borne Organisms. Examination of seed lots to determine their pathological condition has received major attention in Europe and North America. Zybina,

working in Russia in 1927–28, examined 300 samples of flax seed from all parts of the world for the presence of micro-organisms and published the results in 1929. Infection of seeds varied from 4% to 83%, and in the field the degree of seedling mortality correlated well with seed infection except where soil infestation was a factor. On new land he found that 75% of the infection was caused by *Fusarium lini* Bolley and 37% by *Colletotrichum linicolum* Peth. and Laff. Between 1926 and 1930 Doyer in Holland (115) examined commercial seed lots of flax, peas, beet, beans, wheat and barley. She found that 74% of the flax samples were infected in varying degrees with *Botrytis cinerea* f. *lini* (Pers.) and that after two years the fungus had lost its vitality. In one year 84% of the samples were infected with *C. linicolum*. Other organisms detected on seeds were *Ascochyta pisi* Lib. on pea, *Macrosporium* and bacterial infection of bean, *Phoma betae* Frank on beets, *Fusarium* on wheat and *Helminthosporium sativum* P. K. and B., *H. gramineum* (R.) Erick. and *H. teres* Sacc. on barley. This same author in 1934 (116) listed fungi found on clover seed as *Botrytis cinerea* Pers., *B. trifolii, Fusarium, Macrosporium* spp. *Typhula trifolii* Rostr. and *Sclerotinia trifoliorum* Eriks. *A. pisi* (118) was found in varying degrees up to 70% on a large number of pea seed samples. A useful hand book (117) was issued in 1938 which gives the general classification of seed-borne organisms, methods for their detection and suggestions for their control by cleaning and treating. A list of crop seeds with organisms carried by them is included together with illustrations of their symptoms and morphology.

Jorgensen in Denmark examined 89 seed lots of celery seed from Denmark, Germany and France, and reported that 88 of them carried infection with *Septoria apii*. He also found *Phoma rostrupii* Sacc. on carrot seed from plants artificially sprayed when in flower with conidia of the fungus. Muskett and Malone developed the Ulster method for examining flax seed to determine seed-borne parasites by plating, on 2% malt extract agar, from 100 to 500 seeds of a given lot, placing only ten seeds in each petri dish. After incubation for five days at 22° C. they examined the medium around each seed for parasitic organisms. Neergaard (317) made an extensive survey of the seed-borne fungous flora of horticultural crops. He published a list of a large number of plants with the

organisms that may be carried by the seeds together with information as to frequency of seed infection, based largely on his own personal experience with seed testing in Denmark.

Moore examined nearly 30,000 samples of seed between 1925 and 1943 for the presence of seed-borne organisms. About 2% of the samples were rejected because of parasitic organisms and insect pests. Samples of peas, celery and parsley were more commonly rejected than other kinds. Bean seed infected with *Phytomonas phaseolicola* and *Colletotrichum* spp., sunflower seed with *Sclerotium sclerotiorum* (Lib.) Mass. and beet seed with *Uromyces betae* (Pers.) Tul. and *Phoma betae* were also rejected.

Detection of organisms carried by seeds in a North American seed laboratory was undertaken by Munn (292) as early as 1921 when he found spores of *Alternaria cucurbitae* Let. on watermelon seed. Another early attempt to apply phytopathological techniques to seed lots for the detection of seed-borne organisms was initiated in Iowa in 1921 (347). Ear tests of farmers' seed corn in lots of 100 ears from each of more than 100 farmers were used to determine the percentage of ears infected with *Diplodia zeae* (Schw.) Lev. Following the tests by the modified rag-doll method, seed samples of nearly disease-free and of diseased seed were prepared from each lot together with a sample prepared independently by each farmer. All samples were planted with three replications from which stand and yield data were obtained. The results showed that the samples of diseased seed gave the lowest stand and the lowest yield, the nearly disease-free gave the highest, and seed prepared by farmers was intermediate in its behavior. This method of testing seed corn for infection with dry rot fungi was continued and expanded to include work with cereal crops and indexing of onion bulbs suspected of being infected with yellow dwarf virus. Methods of determining smuts of cereal crop seeds and organisms on vegetable crop seeds were described. The results were presented at the Annual Meetings of the Association of Official Seed Analysts in 1931 and 1932 (349, 350), but publication was delayed until 1938. Davis (99) described the haemocytometer method for determining smut spore load in oat seed samples in 1935, and four years later Rice (386) used the same method to measure the fungous spore load in samples of wheat seed. Porter (351) in 1935 described two methods of determining the presence

of *Gibberella saubinetii* in seed lots of barley. One method involved planting the seeds on top of moist blotters on large trays, keeping the trays at 20° C. after which the percentage of infected seeds and of blighted seedlings was determined. The scab fungi grew abundantly from infected seeds and were characterized by a grayish-white or pink color. Equally good results were obtained by planting the seeds in copper boxes containing a mixture of autoclaved sand and soil. When many of the seedlings had reached a height of three to five inches, those produced by infected seed developed seedling blight which was characterized by necrosis of the stem from the seed to a half inch above the surface of the soil. Sporulation of the fungus often occurred on the blighted seedlings. In some cases diseased seedlings emerged only slightly or not at all. Germination of 25 samples in the laboratory correlated well with field performance. As a result of this study it was possible to include, in seed laboratory reports of tests, the percentage of seeds that were diseased and to make recommendations for disease control.

A paper published in 1938 by Porter, Hendershott and Davis indicated the relationships of certain seed-borne organisms to germination in laboratory and field. An examination of 129 seed lots of barley, 123 of corn, 75 of flax, 110 of oats and 21 of wheat was made for the presence of seed-borne organisms that affect germination. The principal organisms detected on cereal seeds were *Gibberella saubinetii, Helminthosporium sativum* and *Fusarium* spp. On corn the dry rot fungi *Diplodia, Fusarium* and *Gibberella* were observed and recorded. Laboratory tests with 200 seeds of each lot were made on top of blotters for observation as to parasitic organisms. An equal number was planted in a mixture of autoclaved sand and soil, and counts were made for percentages of normal seedlings and for those with symptoms of seedling blight. An examination of the seeds of barley, oats and wheat was also made for smut spores. Following the laboratory test, all samples were planted in the field with a number of replications, and the percentage germination was determined. Part of the plantings were made in 1933, but most of them in 1934 when the entire middle western section of the United States suffered from a severe drouth. Field germinations were relatively low, yet the correlation between field and laboratory germination was fairly good, in that samples with a low percentage of germinability caused by seedling blight

fungi in the laboratory gave a correspondingly low emergence in the field. Records of smutted plants that appeared in the field were made also.

In a second paper published in 1938 Porter (357) gave an outline of the classification of organisms and disease-producing entities carried by seeds and vegetative reproductive organs, described the techniques that were being employed in detecting a large number of saprophytic and parasitic fungi on seeds, and listed the effects of different types of organisms on seed germination or on subsequent plant development. A later paper in 1944 (362) gave a partial list of detectable seed-borne organisms with information as to mode of transmission and symptoms on seeds and seedlings.

Crosier has published a series of papers dealing with the examination of seed samples for seed-borne organisms. He outlined methods (84) that were employed in detecting organisms and reported his results in measuring their pathogenicity. One of his first papers (85) described species of *Fusarium* that occurred on pea seed. He concluded that those species were not aggressive pathogens; they caused no wilt and caused root rot or seed decay only when the mycelium was extremely concentrated and free from other organisms. In 1938 this same author (87) issued a report of his examination of 176 samples of oats and barley collected from farmers' drills and granaries. He found smut spores on the seeds of 174 samples and root-rot and seedling blight organisms on nearly half the samples. Crosier and Weimer isolated fungi from seeds of species of *Poa, Agrostis* and *Festuca,* and tested their pathogenicity. The fungi, in order of their importance as contaminants, were *Curvularia spicifera* (Bainier) Boedijn, *Alternaria tenuis* Nees, *Helminthosporium sativum, H. halodes,* species of *Alternaria, Curvularia, Epicoccum, Fusarium, Helminthosporium* and *Phoma.* Additional fungi listed by Crosier (89) as occurring on seeds are species of *Alternaria, Fusarium* and *Helminthosporium* on *Bromus inermis,* a form of *Diplodia* on cotton, species of *Aspergillus, Cephalothecium, Curvularia, Nigrospora, Penicillium, Rhizoctonia* and *Sclerotium* on peanut, species of *Alternaria, Cladosporium, Curvularia, Myriotleciella* and *Stemphylium* on pumpkin, and a species of *Fusarium* and *Coprinus* on peas and spinach, respectively. Those organisms not reported previously on the respective hosts are listed in the table at the end of this section.

Tullis examined rice kernels for the presence of organisms and identified species of 15 genera which he considered of some importance in relation to discolorations and vitality. The genera that he found were *Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Epicoccum, Fukelina, Fusarium, Helococeras, Helminthosporium, Monascus, Nigrospora, Phoma, Podoconis* and *Trichoconis.*

Russell and Ledingham used routine laboratory tests of wheat seed to determine the presence of covered smut and of common root-rot organisms. Embryo exposure which permits entrance of fungi was also determined. Simmonds described a laboratory method by which he could detect loose smut fungi in seeds of barley and wheat. He removed the embryos by treatment with solutions of KOH and NaOH, and either dehydrated, cleared in cedar oil and examined them without staining, or embedded, sectioned and stained them before making a microscopic examination. Since the loose smut mycelia are carried internally, only microscopic examination can detect their presence. The alternative is to germinate the seeds under conditions favorable for growth of the pathogen and either wait for heads to appear or examine the young spikes before they emerge from the boot.

Greaney and Machacek conducted an annual survey from 1936 to 1942 to determine the prevalence, severity and distribution of seed-borne diseases of cereals in Manitoba. They made plating tests of the seed and found that smut fungi and species of *Helminthosporium* were the most common and important. *H. sativum* was common on barley, wheat and rye, *H. avenae* Eidam was prevalent on oats and *H. teres* Sacc. was prevalent on barley. Only 3.4% of the wheat samples carried more than a trace of *Tilletia tritici* (Beij.) Wint. and *T. laevis* Kuhn, but 76% of the oat samples carried spores of *Ustilago avenae* (Pers.) Jens. and *U. laevis* K. and S., and 73% of the barley samples carried spores of *U. hordei* (Pers.) K. and S. and *U. nigra* Tapke.

Ray and McLaughlin isolated six species and varieties of *Fusarium* from cotton seed, and Gordon isolated and classified the species and varieties of *Fusarium* that occurred on farm samples of cereal seeds in Canada consisting of nine species and four varieties. All organisms not hitherto reported are included in the table at the end of this section.

Leukel and Martin studied the effect of several seed-borne organisms on the germination of sorghum seed. They reported that species of *Alternaria, Aspergillus, Fusarium, Penicillium* and *Rhizopus* were found on seed in great abundance and, with the exception of *Aspergillus,* when used as inoculum often reduced emergence and caused subsequent seedling blight, especially at 15° and 20° C. Seeds with seed-coat injuries gave ready entrance to fungi, and even saprophytic forms often reduced emergence, presumably by depleting the food supply or by producing toxins. On the other hand, there are instances, as shown by Henry and Campbell, where fungi, normally parasitic in sterile soil, become inactivated in field soils, presumably by the antibiotic action of soil organisms.

Workers in the Iowa State College Seed Laboratory have made isolations of organisms carried by seeds, and the results have been published in reports of the Iowa Agricultural Experiment Station (378). The principal organisms found were *Fusarium graminearum* Schwabe and species of *Alternaria, Curvuluria, Epicoccum, Gibberella, Helminthosporium, Fusarium, Mucor, Nigrospora* and *Rhizopus* on sorghum, one species each of *Curvularia, Epicoccum, Nigrospora* and *Phoma* on oats, one species each of *Curvularia, Epicoccum, Phoma* and *Trichuris* on timothy, one species each of *Actinomucor* and *Trichuris* on red clover, *Colletotrichum glycine* Hori, one species each of *Alternaria, Fusarium, Gibberella* and *Phomopsis* on soybean and a species of *Fusarium* on *Vicia faba* L.

Detection of the fungus responsible for the blind-seed disease of ryegrass on seed samples before and after harvest has been studied by a number of investigators since 1938. Hyde classified the causal fungus as a species of *Pullularia* and described a method for estimating the percentage of infected seed before harvest and its correlation with seed germinability. He collected a few seed heads from scattered parts of a field and then removed a few spikelets from scattered positions on each spike. Microscopic examination of each floret in the selected spikelets revealed the percentage of infection. The symptoms consisted of (*a*) a sticky fluid which contained the conidiophores, (*b*) a solid, waxy secretion, either clear or pale pink, and (*c*) a rusty-red color of the grain which appeared dark under the diaphanoscope. This method was recommended to determine the advisability of harvesting a field. Rose attempted to determine the percentage of infected seed by conidial number counts.

He placed 100 seeds per sample in a test tube with five ml. of water and heated the tube in a water bath for 15 minutes at 80° C. The tube was then shaken for half a minute, and a drop of the solution was taken and a conidial count made with a haemacytometer. His error of estimate for germination was between 18 and 19, and therefore he concluded that the method could not be recommended for harvest-forecast purposes. He considered Hyde's method superior.

Neill and Hyde (319) placed the blind-seed fungus tentatively in the genus *Helotium* and considered that it was allied to *H. herbarum.* Later (320) they concluded that the name of the causal organism should be *Phialea temulenta* (Prill.) Delacr. Noble found large numbers of spores of what she considered *Pullularia pullulans* (DeBary) Berk. on seeds of *Lolium perenne* and *L. multiflorum* when they were placed in water. Infected seeds possessed very low germinability. She stated that the relationship of the observed fungus to the blind-seed disease in Great Britain was yet to be established, and in the following year Wilson, Noble and Gray (492) found that the blind-seed fungus and *Pullularia* sp. are often found on the same seed. Gray named the causal fungus, tentatively, *Phialea mucosa* Gray. Wilson, Noble and Gray (493) described the symptoms of the blind-seed disease, pointed out its distinction from *Pullularia pullulans,* recorded the fact that at least six fungi showing affinities occur on species of *Lolium* and agreed with Neill and Hyde (320) that *P. mucosa* and *P. temulenta* are identical. Calvert and Muskett studied the blind-seed disease in Ireland and came to the same conclusions as Wilson, Noble and Gray, namely, that *P. temulenta,* the causal fungus, is distinct from *Pullularia pullulans* which was found to be non-parasitic. They also isolated the blind-seed fungus from *Festuca ovina* L., *Agrostis canina* L., *A. palustris* Huds., *Poa pratensis, Holcus lanatus* L. and *Cynosurus cristatus* L. Artificial infection of *Festuca elatior* and *Poa trivialis* L. was also obtained. Fischer found the blind-seed disease to be prevalent in Oregon. The apothecial stage of the fungus was found on *Lolium perenne, L. temulentum* L. and *Hordeum Gussoeanum* Parl. The conidial stage was detected on *Agrostis exarata* var. *monolepis* (Torr.) Hitchc., *Aira caryophyllea* L., *Alopecurus geniculatus* L., *Bromus racemosus* L., *Cynosurus echinatus* L., *Deschampsia caespitosa* P. Beauv., *Danthonia californica* Boland, *Festuca elatior* L., *F. myuros* L., *Glyceria*

borealis (Nash) Batchelder, *H. Gussoeanum, Holcus lanatus* and *Phleum pratense*. Other hosts that have been reported (61) are *Festuca arundinacea* Schreb., *F. rubra* var. *fallax* Hack. and *Lolium multiflorum*.

Meehan (275) made a study of seed samples received by the seed laboratory to determine the organisms carried by seeds of grasses, oats, barley, wheat, clover and soybean. She observed the symptoms of infection on the seeds and seedlings in the germinator, isolated the organisms, identified them and tested their pathogenicity. She made isolations from 25 species of grasses and found 26 species of Dermatiaceae. Of the 271 isolates about 200 belonged to the genus *Helminthosporium* and most of the others to *Curvularia*. *Helminthosporium victoriae* Meehan and Murphy, a new species, was isolated from seedlings of oats (277). Other organisms which Meehan found are included in the list at the end of this chapter. Later (276) she prepared a host index to the seed-borne species of *Helminthosporium* and *Curvularia* on certain grasses.

The percentage of internal infection in seed corn with various seed-borne fungi was found by Koehler and Dungan to be affected by methods of curing the seed. Percentages were 5.1, 18.3 and 69, respectively, for seed lots dried (*a*) four days at 100° F. and 32% relative humidity, (*b*) four weeks at 70° F. and 65% relative humidity and (*c*) three months at 70° F. and 86% relative humidity. Retarded drying markedly increased the infection percentages by *Fusarium moniliforme* Sheldon, *Nigrospora* spp. and *Penicillium* spp.

Another factor that is closely related to the significance of seed-borne fungi is the age of the seed. Shands found that the longevity of *Gibberella saubinetii* in barley seed was only about 18 months, whereas barley embryos under the same conditions of environment remained viable for longer periods. Dungan and Koehler reported that infection of seed corn by *Gibberella zeae* was almost eliminated by one year in storage and that infection by *Nigrospora oryzae* and *Diplodia zeae* was reduced 50% or more. A marked reduction in the infection of *Cephalosporium acremonium* Corin and *F. moniliforme* occurred within three years. On the other hand, the virus of yellow ring spot of tobacco, according to Valleau (472), survived five and a half years in tobacco seed.

Development of techniques for detecting organisms on seeds in

seed testing laboratories may have more far reaching effects in the future than is apparent at present. Seed laboratories receive many kinds of seed each year; hence an excellent opportunity is presented not only to assist in plant disease surveys over a wide area but also to detect the early appearance of new organisms which may become serious if efforts to control them are not launched. To accomplish this possible objective it will be necessary to provide each official seed laboratory with either a trained mycologist or plant pathologist. Seed treatment campaigns have been initiated as a result of seed laboratory tests, thus assisting in disease control. Another possible development is that of determining the presence of seed-borne entities on seed stocks moving in commerce as an aid to plant quarantine regulations. This aspect was discussed by Porter (361) who indicated the types of cooperation between officials of different countries that would be necessary to make such an effort successful. The future holds much promise in both the research and practical aspects of this phase of seed testing.

Lists of Seed-Borne Organisms and Viruses. A valuable compilation of seed-borne organisms including viruses and nematodes was published by Orton in 1931. Since that time a number of investigators have reported additional instances of seed-transmission of disease producing entities. Groves and Skolko, Neergaard (317) and Meehan (275) have described the seed-borne fungi isolated by them, and their publications are of value to others who wish to make comparisons for purposes of identification.

The following list of seed-borne organisms and viruses includes, with a few exceptions, those which have been reported since Orton's publication, insofar as the writer has been able to compile them. The exceptions* are those for which some additional evidence of seed-transmission has been supplied or which have been reported from parts of the world not hitherto included in Orton's list. There are undoubtedly some ommissions which have escaped attention, but it is hoped that the list will be of assistance to phytopathologists and seed analysts.

USE OF SEED FUNGICIDES IN SEED TESTING

Treatment of seeds with fungicides as a part of the practice of seed testing is a recent development which took place almost simultaneously with that of detection of seed-borne organisms. It

SEED-BORNE ORGANISMS AND VIRUSES, AS REPORTED SINCE 1931

Organism or Virus	Host	By Whom Isolated
1. Bacteria		
**Phytomonas aptata* (Brown and Jamieson) Bergey *et al*	Sugar Beet	Ark and Leach
P. incanae Kendrick and Baker	*Methiola incana*	Kendrick and Baker
**P. malvacearum* (E. F. Smith) Bergey *et al*	Cotton	Hare and King
2. Viruses		
Ring spot	Tobacco	Valleau
Yellow ring spot	Tobacco	Valleau
Streak and mosaic	Tomato	Berkeley and Madden
Yellow dwarf	Onion	Henderson
3. Fungi		
Acremoniella atra (Corda) Sacc.	Agricultural seed	Groves and Skolko (160)
A. verrucosa Togn.	Agricultural seed	Groves and Skolko (160)
Actinomucor sp.	Red clover	Rice and Meehan (378)
Alternaria brassicae (Berk.) Sacc.	*Iberis umbellata*	Neergaard (317)
Alternaria brassicae	*Lepidium sativum*	Neergaard (317)
A. brassicae (Berk.) var. *dauci* (Kühn) Bolle.	Carrot	Neergaard (317)
A. cheiranthi (Fr.) Bolle.	Wallflower	Neergaard (317)
A. circinans (Berk. and Curt.) Bolle.	*Iberis umbellata* and radish	Neergaard (317)
A. dianthi Stev. and Hall	Carnation	Neergaard (317)
A. linicola Groves and Skolko	Flax	Groves and Skolko (158)
A. oleracea Milbr.	Cruciferae and *Phaseolus coccineus*	Groves and Skolko (158)
A. porri (Ell.) Neerg.	*Allium* spp.	Neergaard (317)
A. radicina Meier, Dreschler and Eddy	Squash, carrot, parsley	Groves and Skolko (158)
A. raphani Groves and Skolko	Radish	Groves and Skolko (158)
A. rugosa McAlpin	*Schizanthus hybridus*	Neergaard (317)
A. tenuis Nees.	*Poa, Agrostis, Bromus, Festuca*	Crosier and Weimer
A. tenuis	Grasses and agricultural seed	Groves and Skolko (158)
A. tenuissima (Fr.) Wiltsh.	*Godetia hybrida*	Neergaard (317)
A. sp.	Pumpkin	Crosier (89)
A. sp.	Soybean	Tervet

Organism or Virus	Host	By Whom Isolated
A. spp.	Sorghum, soybean	Porter, Rice and Meehan (378)
A. spp.	Rice, sorghum	Tullis, Leukel and Martin
A. sp.	*Gypsophila elegans*	Neergaard (317)
A. sp.	*Zinnia elegans*	Neergaard (317)
Ascochyta caulicola Laub.	Sweet clover	Jones (207)
A. imperfecta Pk.	Alfalfa	Cormack
A. lactucae Restr.	Lettuce	Neergaard (317)
A. pseudo pinodella Bond and Vass.	Pea	Bondortzeva-Monteverde and Vassilievsky
Aspergillus flavus Link	Soybean	Tervet
A. niger Van Tieghem	Soybean	Tervet
A. ochraceous Wilhelm	Soybean	Tervet
A. sp.	Peanut	Crosier (89)
A. spp.	Rice, sorghum	Tullis, Leukel and Martin
**Botrytis anthophila* A. Bond	Red clover	Silow
B. bryssoidea Walk	Allium	Neergaard (317)
B. cinerea Pers.	Cabbage, lettuce, pea, red clover	Doyer (117)
B. cinerea Pers.	*Scorzonera hispanica*, spinach	Doyer (117)
B. cinerea Pers.	*Clarkia elegans, Godetia hybrida*	Neergaard (317)
B. cinerea	*Primula*, rhubarb, zinnia	Neergaard (317)
B. trifolii Van Beyma	Red clover	Doyer (116)
Brachycladium sp.	*Sporobolus* sp.	Meehan (275)
Brachysporium obovatum	*Lolium perenne*	Meehan (275)
Cephalosporium sp.	Red clover	Meehan (275)
Cephalothecium sp.	Peanut	Crosier (89)
Cercospora carotae (Pass.) Solheim	*Daucus* sp.	Thomas
C. zebrina Pass.	Sweet clover	Jones (208)
Chaetomium sp.	Rice	Tullis
Chlamydomyces palmarum (Cooke) Mason	Parsnip, pea	Groves and Skolko (160)
Cladosporium herbarum (Pers.) Lk.	Rice, *Thesium*	Tullis, Chabrolin
C. pisicolum Snyder	Pea	Snyder
Colletotrichum circinans (Berk.) Vogl.	Onion	Neergaard (317)

Organism or Virus	Host	By Whom Isolated
C. glycine Hori	Soybean	Porter, Rice and Meehan (378)
C. lineola Cda.	Sorghum	Porter (357)
C. spinaciae E & H	Spinach	Doyer (117)
Coprinus lagopus Fr.	Spinach	Crosier (89)
Corticium solani (Kuhn) Burt	Pea, spinach	Neergaard (317), Crosier (89)
Curvularia geniculata (Tracy & Earle) Boedijn	Cabbage, flax, pea	Groves and Skolko (159)
C. geniculata	*Agropyron, Avena, Axonopus, Bouteloua,* sorghum and other grasses	Meehan (275)
C. inaequalis (Shear) Boedijn	Flax, grasses, pea, turnip, spinach	Groves and Skolko (159)
C. inaequalis (Shear) Boedijn	Peanut	Prince
C. inaequalis	*Avena, Axonopus, Bouteloua, Paspalum,* timothy and other grasses	Meehan (275)
C. intermedia Boedijn	Timothy	Meehan (275)
C. lunata (Wakker) Boedijn	Pumpkin, rice	Crosier, Tullis
C. lunata	Onion, pepper	Groves and Skolko (159)
C. lunata	*Agropyron, Agrostis, Bouteloua, Chloris, Dactylis,* rice and timothy	Meehan (275)
C. maculans (Bancroft) Boedijn	Rice	Tullis
C. maculans	Timothy	Meehan (275)
C. pallescens Boedijn	Cauliflower, turnip	Groves and Skolko (159)
C. pallescens	*Axonopus*	Meehan (275)
C. spicifera (Bainier) Boedijn	*Poa, Agrostis, Festuca*	Crosier and Weimer
C. spicifera	*Avena, Axonopus, Bouteloua, Buchloe, Chloris, Dactylis, Hordeum, Paspalum, Triticum*	Meehan (275)
C. trifolii (Kauffman) Boedijn	Timothy	Meehan (275)
C. sp.	Peanut	Crosier (89)
C. sp.	Sorghum, timothy	Porter, Rice and Meehan (378)

Organism or Virus	Host	By Whom Isolated
Dendryphium penicillatum (Corda) Fr.	Poppy	Neergaard (317)
D. spp.	Poppy, meadow fescue	Doyer, Meehan (275)
Didymella lycopersici Kleb.	Tomato	Alcock
Diplodia natalensis Evans	Peanut	Prince
Epicoccum neglectum Desm.	Rice	Tullis
E. purpurascens Ehrbg.	*Poa, Agrostis, Festuca*	Crosier and Weimer
E. sp.	Sorghum, timothy	Porter, Rice, Meehan (378)
Erysiphe cichoracearum DC.	Zinnia	Baker and Locke
E. sp.	Parsley	Doyer (117)
Fusariella sp.	Peanut	Prince
Fusarium avenaceum (Fr.) Sacc	Carrot	Neergaard (317)
F. chlamydosporum Wr. and Rg.	Cotton	Ray and McLaughlin
F. chlamydosporum	Peanut	Prince
F. concolor Rg.	Barley	Gordon
F. culmorum (W. Sm.) Sacc.	*Poa, Agrostis, Festuca*	Crosier and Weimer
F. equiseti (Cda.) Sacc.	Cotton	Ray and McLaughlin
F. graminearum Schwabe	Cereals	Gordon
F. graminearum	Sorghum	Porter, Rice, Meehan (378)
F. lateritium Nees.	Peanut	Prince
F. lateritium var. *uncinatum* Wr.	Peanut	Prince
F. lycopersici Brushi	Tomato	Kendrick (222)
F. moniliforme Sheld	*Poa, Agrostis, Festuca*	Crosier and Weimer
F. moniliforme	Peanut	Prince
**F. niveum* E. F. Sm.	Watermelon	Taubenhaus
F. orthoceras App. and Wr. var. *pisi* Linford	Peas	Snyder
F. oxysporum Schlecht.	Cereals	Gordon
F. oxysporum	Peanut	Prince
F. oxysporum	Peas	Crosier (89)
F. poae (Pk.) Wr.	Cereals	Gordon
F. reticulatum Mont.	Peanut	Prince
F. sambucinum Fuck. var. *coeruleum* Wr.	Cereals	Gordon
F. sambucinum form 6	Durum wheat	Gordon
F. scirpi Lamb. and Fautr.	Cotton	Ray and McLaughlin

Organism or Virus	Host	By Whom Isolated
F. scirpi Lamb. and Fautr. var. *acuminatum* (Ell. and Ev.) Wr.	Cotton	Ray and McLaughlin
F. scirpi Lamb. and Fautr. var. *acuminatum* (Ell. and Ev.) Wr.	Cereals	Gordon
F. scirpi Lamb. and Fautr. var. *caudatum* Wr.	Poppy	Christoff
F. scirpi Lamb. and Fautr. var. *compactum* Wr.	Cereals	Gordon
F. semitectum Berk. and Rav.	Cereals	Gordon
F. semitectum	Cotton	Ray and McLaughlin
F. solani (Mart.) App. and Wr.	Cotton	Ray and McLaughlin
F. solani f. *eumartii* (Carp.) Wr.	Peanut	Prince
F. sporotrichoides Sherb.	Cereals	Gordon
F. tracheiphilum E. F. Smith	Cowpea	Kendrick (223)
F. vasinfectum Atk.	Okra	Taubenhaus
F. sp.	*Bromus inermis*	Crosier (89)
F. sp.	Cantaloupe	Leach
F. sp.	Lettuce	Doyer (117)
F. sp.	Red clover	Doyer (117)
F. sp. (wilt)	Beans	Kendrick
F. sp.	Sorghum	Porter, Rice and Meehan (378)
F. sp.	Sorghum	Leukel and Martin
F. sp.	Soybean	Porter, Rice and Meehan (378)
F. sp.	Soybean	Tervet
F. sp.	*Vicia Faba*	Porter, Rice and Meehan (378)
F. spp.	Peanut	Prince, Wilson (489)
F. spp.	Rice, peanut	Tullis, Wilson (489)
F. spp.	Spinach	Doyer (117)
***Fukelina* sp.**	Rice	Tullis
***Gibberella* sp.**	Sorghum	Porter, Rice and Meehan (378)
G. sp.	Soybean	Porter, Rice and Meehan (378)
Gliocladium catenulatum Gilman and Abbott	Peanut	Prince
Helicoceras oryzae Linder and Tullis	Rice	Tullis
Helminthosporium catenarium Drechsl.	*Buchloe, Festuca,* timothy	Meehan
H. dictyoides Drechsl.	*Buchloe, Festuca*	Meehan

Organism or Virus	Host	By Whom Isolated
H. halodes Drechsler	*Poa, Agrostis, Festuca*	Crosier and Weimer
H. oryzae Van Breda de Haan	*Festuca*, timothy	Meehan (275)
H. rostratum Drechsl.	*Axonopus, Bouteloua, Chloris Cynodon, Paspalum*, timothy	
H. sativum P. K. and B.	*Poa*	Meehan (275)
H. sativum	Bromegrass	Crosier (89)
H. triceptatum Drechsl.	*Agropyron, Buchloe*, timothy, sorghum, *Dactylis*	Meehan (275)
H. turcicum Pass.	*Agrostis*, timothy	Meehan (275)
H. victoriae Meehan and Murphy	Sudan grass	Chilton (66)
H. sp.	*Agropyron, Avena, Chloris, Dactylis, Festuca, Paspalum*, timothy, *Setaria*, sorghum	Meehan (275)
Leptophaeria pratensis Sacc. and Briard	Sorghum	Porter, Rice and Meehan (378)
Macrophomina phaseoli (Maubl.) Ashby	Sweet clover	Jones (208)
Macrosporium sp.	Bean	Andrus
Miriotleciella sp.	Red clover	Doyer (117)
Monascus purpurescens Went, Hedw.	Pumpkin	Crosier
Mucor sp.	Rice	Tullis
M. sp.	Sorghum	Porter, Rice and Meehan (378)
Mycosphaerella lethalis Stone	Peanut	Prince
Nigrospora oryzae (B. and Br.) Petch	Red clover	Jones (208)
N. sp.	Rice	Tullis
N. sp.	Peanut	Crosier (89)
Penicillium sp.	Sorghum, oats	Porter, Rice and Meehan (378)
P. sp.	Sorghum	Leukel and Martin
P. sp.	Soybean	Tervet
Peronospora arborescens (Ber.) de Bary	Peanut	Prince
P. effusa (Grev.) Rbh.	*Meconopsis*	Alcock
P. parasitica (Pers.) Tul.	Spinach	Cook
**P. schachtii* Fckl.	Cabbage	Alcock
P. viciae Berk.	Beet	Leach (242)
	Pea	Alcock

Phialea temulenta (Prill.) Delacr.	*Lolium* and other grasses	Gray, Neill and Hyde, Calvert and Muskett, Fischer
Phoma glomerata (Cda.) Wright and Hochapfel	Grasses	Crosier (89)
**P. insidiosa* Tassi	Sorghum	Porter (357)
P. nemophilae Neerg.	*Nemophila* spp.	Neergaard (317)
P. rhoedis Brun.	Poppy	Neergaard (317)
**P. restrupii* Sacc.	Carrot	Jorgensen
P. siliguae Sacc.	Wallflower	Neergaard (317)
P. sp.	Spinach	Doyer (117)
P. sp.	*Valerianella olitaria*	Doyer (117)
P. spp.	Rice	Tullis
Phyllosticta acetosa Sacc.	*Rumex*	Neergaard (317)
P. primulicola Desm.	*Primula*	Neergaard (317)
Phytophthora infestans (Mont.) De By.	Tomato	Boyd
Pleospora calvescens (Fr.) Tul.	Poppy	Christoff
P. herbarum (Pers.) Rbh.	Bean, onion	Doyer, Alcock
P. herbarum	Many agricultural seeds	Groves and Skolko (157)
Podoconis sp.	Rice	Tullis
Puccinia porri (Sow.) Winter	*Allium*	Alcock
Pullularia pullulans (De Bary) Berk.	*Lolium*	Noble
Ramularia sp.	*Parthenium argentatum*	White
Rhizopus artocarpi Rac.	Peanut	Prince
R. solani Kühn	Subterranean clover	Chilton (67)
R. solani	Peanut	Prince
R. sp.	Peanut	Crosier (89)
R. spp.	Peanut	Wilson
Rhizopus nigricans Ehbg.	Peanut	Prince
R. sp.	Sorghum	Leukel and Martin
R. sp.	Peanut	Prince
Sclerochaeta sp.	Peanut	Prince
***Sclerospora graminicola* (Sacc.) Schroet.	Millet	Porter (357)
Sclerotinia sclerotiorum (Lib.) Schroet.	Bean, *Scorzonera hispanica*	Doyer (17)
S. sclerotiorum	Carrot, dahlia	Alcock

S. sclerotiorum	Pea	Crosier (89)
S. trifoliorum Eriks.	Red clover	Doyer (117)
Sclerotium bataticola Taub.	Peanut	Prince, Wilson (489)
S. sclerotiorum (Lib.) Mass.	Sunflower	Moore
Septoria petroselini Desm.	Parsley	Doyer (117)
Sphacelotheca reiliana (Kuehn) Clint. (*Sorosporium reilianum* (Kuehn) McAlpine)	Corn	Bressman and Barss.
Sphaeronema sp.	Cabbage	Doyer (117)
Stagonospora meliloti (Lasch.) Petr.	Sweet clover	Jones (209)
Stagonosporopsis hortensis (Sacc. and Mulbr.) Petr.	Bean	Doyer (117)
Stemphylium consortiale (Thüm) n. combr.	Many agricultural seeds	Groves and Skolko (157)
S. radicinum (M. D. and E.) Neerg.	Carrot	Doyer (117)
S. radicinum	Celery	Neergaard (317)
S. radicinum var. *petroselini* Neerg.	Parsley	Neergaard (317)
S. sarcinaeforme (Cav.) Wilts.	Red clover	Chilton
S. sp.	Pumpkin	Crosier
Trichocladium aspermum (Cda.) Harz.	Carrot, lettuce, pea, pumpkin, *Vicia Faba*	Groves and Skolko (160)
Trichoconis caudata (Ap. and Str.) Clem.	Rice	Tullis
Trichoderma viride Fr.	Peanut	Prince
Trichuris spp.	Red clover, timothy	Porter, Rice and Meehan (378)
Typhula trifolii Rostr.	Red clover	Doyer (117)
Uromyces betae (Pers.) Tul.	Beet	Alcock
Ustilago tragopogi-pratensis (Pers.) Rouss.	Salsify	Alcock
Verticillium sp.	Eggplant	Kadow

is natural that these two aspects of seed technology should have been emphasized together, but the rapid development of dust fungicides no doubt accelerated their use in seed testing. It is not the purpose of this chapter to review the extensive literature dealing with the widespread use of seed disinfectants as one method of disease control, but rather to review only those papers which bear, more or less directly, on seed testing in both its research and practical aspects. Developments in this field have included (*a*) laboratory and field germination of treated and untreated seed known to be infected with specific organisms and (*b*) treatment with disinfectants to observe their effect on parasitic and saprophytic organisms carried by seeds. These two phases are treated together.

A beginning in the use of seed disinfectants in connection with seed testing was made in 1928 (349) when samples of farmer's seed corn were ear-tested and classified as nearly disease-free or infected with dry rot fungi. A third class was prepared by visually selecting, from early picked seed, ears that appeared relatively free from disease. All three classes were then divided into two subclasses, one treated, the other untreated, and all were planted in the field where counts for germination were made and yields were recorded. The results showed that visually selected treated seed yielded as well as nearly disease-free seed either treated or untreated. These results were presented at the Annual Meeting of the Association of Official Seed Analysts in 1930, but publication was delayed until 1938. This work with farmers' seed corn marked the beginning of an intensive educational campaign in Iowa for early selection, careful drying, dry storage, ear selection followed by a representative germination test and treatment of seed corn with mercury dust fungicides as a substitute for wholesale ear testing.

Further studies were initiated in 1932 and 1933 with seeds of barley, corn, flax, oats and wheat, from which several publications followed. The first one dealt with laboratory and field germination of barley seed (351) infected with *Gibberella saubinetii* and *Helminthosporium sativum*. Untreated and treated seeds of lots with varying degrees of infection were planted on blotters, in a mixture of sand and soil and in the field. The percentages of infected seeds and seedlings, of normal sprouts and of blighted seedlings were recorded from the laboratory tests. Percentage germination in the field was determined and recorded. One interesting feature of

the investigation was the use of 5% ethyl mercury phosphate (New improved Ceresan) diluted with talc to make a 1% dust. Treatment with the ethyl mercury phosphate was thus facilitated by applying a slight excess of the talc-phosphate mixture and removing the excess by shaking the treated seed in a tea strainer. The higher percentage of germination from treated than untreated seed obtained in the laboratory was duplicated in the field.

The results of subsequent tests published in 1938 (366) involved seeds of barley, corn, flax, oats and wheat, a total of 458 samples of farmers' seed infected in varying degrees with seed-borne organisms. In most cases there was a good correlation between the germination of samples in the field and laboratory, and the higher percentage germination of treated than untreated seed was relatively maintained under both sets of conditions. Three types of diluents for 5% ethyl mercury phosphate were used, namely, talc, gypsum and button dust. Gypsum alone exhibited some germicidal value on cereal seeds infected with *G. saubinettii*. Yield results from all treated and untreated seed lots were also obtained. The data resulting from this study illustrated the applicability of such pathological techniques as detection of seed-borne organisms and the effect of disinfectants on seed germination to seed laboratory practice, in that the laboratory tests were reasonably indicative of field response.

In a paper (353) presented at the Annual Meeting of the Association of Official Seed Analysts in 1936 the use of seed disinfectants in seed laboratory practice was described as a practical measure for teaching an important method of disease control to recipients of seed laboratory reports. This was accomplished by stating the germination of treated and untreated seed on the same report, as well as the percentage of diseased seeds or seedlings for such crops as barley, corn, flax, oats, peas and wheat. The dusts were applied in the laboratory by placing the seeds with the proper amount of dust in a bottle which was rotated on two rollers, one of which was driven by an electric motor. Mercury poisoning, which results from an excess of volatile mercury compounds applied to seeds, was also illustrated. Sass found that over-doses of ethyl mercury phosphate applied to certain seeds caused giant nuclei which resulted in some lateral growth but no elongation of the plumule.

A possible classification of seed fungicides, their use in seed research and seed laboratory practice for the control of parasitic and saprophytic organisms and their effect on seed germination in laboratory and field was published in 1938 (357). The seeds that were studied included lima beans, peas, sorghum and soybean, in addition to those previously studied.

Arasan, Ceresan and Spergon were applied to five lots of pea seed, and Arasan, Semesan J. and Spergon were applied to 22 lots of sweet corn and six lots of popcorn planted on cellucotton or blotters (364). All treatments reduced the percentage of decayed peas, gave almost complete control of a species of *Fusarium* and largely eliminated infection by saprophytic fungi. The treatments applied to sweet corn gave good control of *Fusarium* spp. and fairly good control of saprophytes, but Arasan was superior to both Semesan Jr. and Spergon. The percentages of seed infected with saprophytic forms were 67, 13.1, 21.7 and 22.7 for untreated and Arasan, Semesan Jr. and Spergon treated, respectively. On popcorn seed all three treatments gave satisfactory control of parasitic and saprophytic fungi.

Crosier and his associates in New York have made a series of contributions toward the use of disinfectants in a seed laboratory for the control of parasitic and saprophytic fungi. One of the earliest papers described the abnormal germination of wheat seed caused by organic mercurials (86). Crosier and Nelson used Barbak C., Ceresan, $HgCl_2$ and New Ceresan on lima bean seed with resultant control of contaminating micro-organisms during the germinating period. Crosier and Patrick (92) investigated the effect of hot water and chemical dusts on seeds of cabbage, radish and broccoli infected with *Alternaria* spp. They found that the beneficial action of seed treatment is dependent on the amount of seed contamination by both *Alternaria* and *Rhizopus*. The mercurial disinfectants effected partial control, but Cu_2O and ZnO were less effective. Removal of excess mercurial dusts from the seeds was considered necessary. Crosier (88) treated seed of cucumber, muskmelon and squash, prior to germination, with Spergon, United States Rubber Co. materials No. 601 and No. 604, and with the ethyl group of mercurials. Spergon was excellent for the control of damping off, but was considered a poorer fungicide than the ethyl mercury compounds. A referee test among 11 official seed testing stations

(90) was conducted, using pea seed and Stowell's Evergreen sweet corn seed infected with *Diplodia zeae* and other mold fungi, treated with various chemicals in comparison with untreated seed. Arasan excelled in the control of *Diplodia zeae* and Fusaria; and No. 1452 C in the control of *Rhizopus nigricans,* in seed germination and in green weights of seedlings of sweet corn. Arasan was superior to Semesan Jr., Ceresan, Spergon and No. 604 in the increase over untreated seed of green weight of seedlings. Spergon proved superior in the control of *R. nigricans* and in percentage of germinated seed, and No. 604 gave better control of bacteria and other mold fungi. Crosier and Patrick (93) reported that Arasan alone or mixed with flour applied to ten lots of sweet corn seed retarded the growth of *D. zeae,* Fusaria and *R. nigricans* and that the seedlings from Arasan-treated seed weighed more than those from untreated seed.

Doyer in Holland has reported the use of seed disinfectants in connection with seed testing. She treated flax seed (115) with Tillantin R. which gave satisfactory control of *Botrytis cinerea* f. *lini* and *Colletotrichum linicolum.* The compound U. T. 685 dusted on beet seed controlled *Phoma betae.* Clover seed (116) infected with species of *Botrytis, Macrosporium, Sclerotinia* and *Typhula* was treated with Germisan and Ceresan which gave good control. Various seed-borne diseases of vegetables were described in 1941 (118), and methods of seed disinfection were given. In one instance untreated lettuce seed with a germination of only 29% gave 76% germination after treatment with Ceresan.

One of the most practical results of treating seeds prior to a germination test is that it can promote more uniformity in the evaluation of seed lots. When samples are overrun with saprophytic organisms or severely infected with parasitic species it becomes difficult to evaluate a germination test. Yet this difficulty can usually be overcome by proper seed treatment. A referee test (379–1938: 52) conducted in 1938 with treated and untreated seed of barley and wheat demonstrated in a remarkable way the uniformity of interpretation of normal seedlings by five different stations when the seed was treated with ethyl mercury phosphate. Evaluation of seedlings produced by the untreated seed was highly variable. On the other hand, no laboratory should report a test of treated seed without a corresponding test of untreated seed un-

less the sample was treated before the laboratory received it. The reason for this precaution is obvious.

Finally the ultimate application of seed disinfection to seed testing is dependent on the extent to which commercial lots of seed are treated with disinfectants as a part of seed processing prior to sale. There is a growing need and demand for treated seed of many field and vegetable crops. As more non-poisonous compounds become available and mailing restrictions are modified, more and more seed will be sold as treated seed. Seed laboratories that receive untreated samples which are to be marked as treated seed will be justified in treating a portion of the sample prior to making a viability test. Familiarity with different seed treatment compounds will thus become a further necessary requirement of seed technologists.

DEVELOPMENT OF LABORATORY METHODS FOR MEASURING SEED GERMINABILITY UNDER ADVERSE CONDITIONS

The discussion in Chapter 5 of this paper pointed out that the criterion for measuring seed germinability has been the percentage of normal seedlings which are produced under optimum conditions when such are known for each kind of seed. It has been recognized that field germination, except under a very favorable environment, cannot be expected to equal that in the laboratory. Three developments within the past few years have served to indicate the desirability of knowing in advance of planting how certain kinds of seed may be expected to germinate in an environment considerably below the optimum. In the first place there has been a pronounced interest among phytopathologists in seedling blight and damping-off caused by soil-borne fungi. A study of these conditions has revealed valuable information relative to the interrelationship of the temperature, moisture and fungous flora of the soil in their effect on germinating seeds and on seedlings. In the second place the marked production of new seed protectants has made it necessary to develop laboratory methods for their evaluation. In the third place plant breeders have produced a tremendous number of new crop varieties by hybridization, especially of field and sweet corn. This program has not only created innumerable genetic combinations in crop plants but has also resulted in large scale commercial production of expensive seed stocks

that have required artificial drying and more mechanical processing methods than were previously employed. Field experience, especially earlier planting than in previous years, has revealed striking differences in germinability of seed stocks which were not detectable under favorable laboratory conditions.

It is neither the purpose nor function of this present paper to review the voluminous literature dealing with soil fungi, the innumerable field tests with seed fungicides or the production of new varieties and hybrids but rather to limit the review to a few publications which have a pertinent relationship to this new phase of seed laboratory practice. This review covers not only the germination of untreated seed under adverse conditions in laboratory and field but also the comparative value of seed protectants under the same conditions.

Horsfall has made valuable contributions to our knowledge of the factors that determine the extent of pre-emergence and post-emergence damping-off of seedlings as well as the relative benefits of several seed protectants. In 1932 (190) he published his results with red oxide of copper (Cu_2O) as a seed treatment to combat damping-off of several kinds of vegetable crop seedlings. He made plantings in greenhouse flats, using soil infested with *Pythium ultimum* Trow. Cu_2O was found to be especially valuable for smooth seeds such as pepper and egg-plant because of its adhesive qualities, and on tomato seeds it adhered two and a half times as well as monohydrated copper sulfate. On the basis of copper content the adhesiveness was seven times as great. His paper published in 1938 (191) summarized nine years of work on damping-off and its control by seed protection and by soil treatments. One of the important conclusions was that high soil moisture seems to enhance pre-emergence damping-off more than low soil temperatures. He also investigated inoculum potential or disease-producing power of the soil in relation to seed protection. When the inoculum potential exceeded 80% of the maximum the protective power of Cu_2O and $CuSO_4$ fell off rapidly. He calculated that 0.5 mg. of metallic copper (as cuprous oxide) per sq. cm. of seed surface is required to give minimum coverage dosage on seed, without regard to its kind.

The comparative germination of treated and untreated pea seed in auto-claved and non-auto-claved soil was studied by Davis (101)

who found a marked difference in seedling emergence from untreated seed planted in the two kinds of soil. In non-auto-claved soil the percentage emergence was severely reduced by the attack of soil fungi, and seed treatment provided an effective control against the seed-rotting organisms. Ogilvie *et al* treated peas with Cu_2O and found that under certain environmental conditions in the greenhouse and field it increased the emergence of seedlings, although it sometimes retarded the rate of emergence. In dry soils Cu_2O caused injury to seed of some varieties.

Reddy and Rice reported that seed corn which gave high germination under favorable conditions in the laboratory often germinated much less when planted in moist unsterilized soils kept at a low temperature. Seed treatment proved beneficial under adverse conditions. A tensiometer designed by the junior author was of value in regulating the amount of water in the soil for any specific test. Tatum and Zuber used the method developed by Rice, as employed in the Iowa State College seed laboratory, to test the germination of corn that had received different processing treatments. Their results showed that under adverse conditions (low temperature in non-sterile soil) marked injury to germination of seed corn occurred. Machacek and Wallace considered that non-sterile soil served as a good medium in which to test the response of cereal seeds prior to field planting. The results obtained were considered more indicative of probable field performance. Leukel and Martin used soil artificially infested with several species of soil- and seed-borne fungi to test the germination of sorghum seed.

Rice (387) made an extensive investigation of the germination of many field crop seeds in *Pythium*-infested soil mixed with different amounts of sand. He varied the temperature and the amount of water that was maintained in the soil and planted untreated seed and seed treated with many different commercial fungicides. Samples of corn of different ages and freshly-harvested corn dried by different methods as well as other crop seds were used in his investigations. Planting dates in the field varied from very early to the normal planting time. He used a tensiometer designed by himself and produced in the College Instrument Shop to measure the water tension in soil which served as an indicator of moisture content. His results showed that (*a*) the moisture content

of *Pythium*-infested soil mixed with sand had a marked effect on the percentage of germination and normal seedlings of different kinds of field crops, (*b*) the length of time of exposure of seeds to low temperatures was an important factor in determining the amount of seed decay and damping-off, (*c*) exposure of corn, soybean, sorghum and flax seed at 10° C. in *Pythium*-infested soil for five to ten days followed by a more favorable temperature gave a fair indication of germination under adverse conditions, and (*d*) the relative protective power of seed fungicides could be measured by treating seeds and planting them in moist soil kept at 5° to 10° C. for a part of the germination time. In an experiment station report (362) in 1943 the mean percentage germination of 177 lots of hybrid corn tested in sterile sand at a daily temperature range of 68° to 86° F. was reported as 92. The same samples germinated in *Pythium*-infested soil and sand at 10° C. for seven days followed by a temperature of 26° to 28° averaged 67.5% germination.

Leukel tested 19 materials on a number of varieties of sorghum seed to determine their effect on seedling emergence in sterilized and non-sterilized soil and in the field. He concluded, among other things, that soil temperature and moisture have an important influence on the emergence of seedlings from treated and untreated seed. In general, when seeds were planted in soils infested with virulent fungi, the absolute increase in emergence from treated seed was greatest at 20° C. and least at 15° C., whereas the relative increase was greatest at 15° C. and least at 25° C.

Leach (244) concluded that growth rates of host and pathogen are primary factors that determine the severity of pre-emergence damping-off. Leach and Smth suggested that emergence rates could be measured by calculating a mean emergence period. In their work with seed protectants for peas they stated that comparisons of emergence rates should be limited to trials in sterilized soil or between treatments having equal protective effects. They showed that in pasteurized soil none of the seed treatments tested on disease free pea produced more rapid germination or larger seedlings than the non-treated controls; in fact, yellow cuprous oxide not only delayed emergence and water absorption by the cotyledons but produced necrotic areas on the surface of the cotyledons. In soils heavily infested with *Pythium ultimum,* Semesan, yellow copper oxide and dichloro-naphtha-quinone were among the

most effective materials used for the control of pre-emergence damping off. The benefits of seed treatment were considered as entirely due to disease prevention.

Taylor and Rupert used *Pythium*-infested soil in which to plant vegetable seeds in greenhouse flats. They used treated and untreated seed and observed the healthy and damped-off seedlings. Arasan and Fermate gave better control of pre-emergence and post-emergence damping-off than Cuprocide, Semesan and Spergon.

A recent publication by Porter (364) contains a considerable body of data bearing on the germination of vegetable crop seeds under adverse conditions in both laboratory and field. Two methods of testing seed samples were employed in the laboratory, one of which involved optimum, the other adverse conditions. The latter method was basically the same as described and used by Rice (387) in that the laboratory medium consisted of two parts sand and one part *Pythium*-infested soil, and the temperature used was 10° C. for different periods of time, depending on the kind of seed. The tests under optimum conditions were made to observe the seeds and seedlings for symptoms of parasitic and saprophytic organisms as well as to determine maximum viability. The tests under adverse conditions employed both treated and untreated seed, and plantings were made in the field to compare the results with those in the laboratory. In many cases field conditions were adverse for germination, but not in every instance. This investigation involved 50 samples of peas, 41 of sweet corn, six of popcorn, five of soybean, 14 of spinach, 11 of onion, six of beet, five of carrot, one of tomato and nine of bush lima and green pod beans. The data obtained by this study revealed that by planting untreated and treated vegetable seeds under standardized adverse conditions it was possible to,

(*a*) index samples and varieties as to their resistance or susceptibility to seed decay and damping-off;

(*b*) measure the relative resistance to adverse germinative conditions of old and new crop seed of the same variety, which gives an indication of physiological decline in vitality resulting from age of seed;

(*c*) obtain a fair index of expected germination of seed lots planted under adverse field conditions;

(*d*) index the relative effectiveness of seed protectants for the control of seed decay and damping off.

This particular study and a previous article (363) also revealed that the cold soil treatment may induce a pathological type of baldhead in soybean and lima bean seedlings, a condition which resembles that attributed to mechanical injury of seed during threshing (46, 168). Arasan provided an effective control of pathological baldhead in cold soils.

APPLICATION OF STATISTICAL METHODS TO SEED TESTING

The application of statistical methods to the design of experiments and to the analysis and interpretation of biological data has received unusual emphasis the past 15 to 20 years. Fortunately the science of seed testing has been included in this emphasis, particularly within the past decade. Snedecor, by his text book, has provided a useful guide, and Leggatt (251, 252) has made major contributions to this important aspect of seed testing. Others have demonstrated some of the practical applications of statistical science to seed technology.

Leggatt's review (252) is of particular significance because it deals specifically with problems in seed technology from both the theoretical and applied standpoints. A comprehensive review of his paper is unnecessary. His major contributions have dealt with:

(*a*) distribution of small, rough and smooth seeds in bulk lots with different sizes and seed coat characteristics from which he has been able to estimate the size of sample necessary to determine, on a practical basis, the distribution of weed seeds in crop seed or the percentages of different kinds of seeds in mixtures;

(*b*) evaluation of germination tests made by different analysts or by one analyst who compared two or more methods of testing one sample or one method with different samples; he prepared a set of tables to be used in comparing differences between replicate tests when a different number of seeds were used in each replicate;

(*c*) evaluation of different methods of sampling seeds in bags with the objective of obtaining representative samples for analysis;

(*d*) preparation of tolerance tables to be used in making comparisons between replicate purity analyses from a given lot of seed or in estimating the reliability of separate reports on the rate of occurrence of weed seeds in a given lot.

Woodbridge studied the distribution of the seeds of *Cirsium arvense, Rumex crispus* and *Barbarea vulgaris* in timothy and con-

cluded that the latter two became consistently distributed according to the Poisson series, but seeds of *C. arvense* did not. She concluded that 50 grams of timothy seed was needed to determine the content of the three weed species within allowable limits if the number exceeded 30 per 50 grams.

Porter and his associates (354, 356, 358, 362, 366) have applied the chi-square test for homogeneity, the analysis of variance and the coefficient of variability extensively in their analyses of laboratory and field data. These techniques have been of particular value in (*a*) determining the degree of natural variation in purity of small sub-samples drawn from a well mixed lot, (*b*) measuring the homogeneity of large lots after mechanical mixing has occurred, (*c*) measuring the accuracy and uniformity of separation obtained with an air-blast seed separator for the analysis of replicate samples of seed, and (*d*) determining the maximum differences that may be expected between replicate samples of seed tested for germination.

These applications of statistical methods to the practice of seed testing have not only illustrated the importance and value of developing more standard and uniform procedures but have led to the adoption of standardized methods. In addition they have contributed to more effective and equitable enforcement of seed laws.

More recently Leggatt (255) has applied the principles of "Sequential Analysis" to the problems of seed testing. The advantages of this type of analysis are considered to be that the "quality of a product can be maintained with approximately one-half the sampling that is necessary by other methods of quality control or, alternatively, that with the same amount of sampling the degree of certainty or quality control may be increased or we may say that the limits of variability may be reduced". The procedure of Sequential Analysis, as described, "is to draw a number of samples and test them sequentially one after another basing a decision as to whether the lot is acceptable or unacceptable, or whether further testing is required, upon the total quantity of material analyzed up to that particular sample". He prepared a series of tables to be used in determining the proper decision in connection with counts from weed seeds in seed lots. The use of this principle of analysis requires the establishment of the "Acceptable Quality

Limit" and the "Unacceptable Quality Limit". It is probable, as suggested by Leggatt, that the application of "Sequential Analysis" to the science of seed testing will greatly aid in reducing the pressure of work and the size of test in relation to accuracy of results obtained.

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Since proofreading of this article in August, 1947, while the writer was on leave of absence in the United States before returning to Brazil, a number of papers have appeared to which reference might well be made. To do so would necessitate rewriting a portion of the article, which, of course, can not very well be done. Three papers of interest and related to two phases discussed in the article are *a*) Distinguishing the species of *Brassica* by their seed. Albina F. Musil, U. S. Dept. Agr., Misc. Publ. 63. 1948; and (*b*) the papers on seed treatment by R. W. Leukel and J. C. Walker in recent issues of THE BOTANICAL REVIEW.

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